NUCLEAR SIZES IN RANA MESONEPHROI [1]

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When Jacobj (1925) devised the now classical caryometric method there was opened up for the cytologist a whole new field in population dynamics. Jacobj demonstrated that for any given organ the nuclear population showed considerable variation in size from one individual nucleus to another. By plotting frequency distribution curves of nuclear volumes he obtained evidence to show that the volumes increased discontinuously according to a logical pattern. The peaks of the curve corresponded to nuclear volumes which when arranged in series gave the geometric progression 1:2:4:8. Nuclear class series have since been described for a wide range of both invertebrate and vertebrate tissues.

Caryometry has been used extensively in investigations of ploidy, of endomitotic growth, and of the interphasic growth of nuclei in a dividing tissue. The concept that nuclear size is a function of ploidy has proved fruitful in the study of ploidy in amphibians (*cf.* Fankhauser, 1945; Gallien, 1953). This idea was used advantageously in the interpretation of polymodal curves obtained from nuclear volume data derived from studies of the kidneys of frogs: it was indicated that polysomaty may occur in this organ (Dawson, 1948; Schreiber and Melucci, 1949). Polysomaty or endopolyploidy is understood in this paper as that condition existing in a normal diploid somatic tissue in which there is a certain percentage of polyploid cells and/or polytene chromosomes. Furthermore, the concept that nuclear size is a function of chromosomal reduplication has been helpful in the interpretation of data having to do with interphasic growth of nuclei in a dividing tissue. Nuclear class series indicative of a mitotic cycle have been described in *Ambystoma* larvae (Swift, 1950) and in *Rana pipiens* embryos (Sze, 1953). Both of these investigations were primarily concerned, not with relationships in size, but with the photometric determination of amounts of desoxyribose nucleic acid (DNA) in interphasic nuclei. The introduction into cytology of photometric techniques has renewed interest in the caryometric interpretations of nuclear sizes.

Nuclear size as a reflection of ploidy relates importantly to amphibian development both in regard to gross morphology and in regard to tissue differentiation (Fankhauser, 1945; Gallien, 1953). Also it has been shown in certain molds that both cell size and nuclear size changes may accompany morphogenesis (Bonner, 1957). Furthermore, it has been hypothesized that DNA may show a slight decrease with the progressive differentiation of certain *R. pipiens* tadpole tissues (Moore, 1952). Also of importance is the fact that nuclear size may be related to the degree of functional activity of the cells in question. For instance, spinal

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cord cells of *R. temporaria* show a decrease in volume following narcosis (Krantz, 1947). On the other hand, nuclear size may increase as a result, not of chromosomal increase, but as a result of protein synthesis in the nucleus. This has been shown to be true for certain insect and mammalian tissues by Schrader and Leuchtenberger (1950) and by Leuchtenberger and Schrader (1951).

Nuclear size is, then, of considerable importance in the investigations of some of the most fundamental biological problems. The concepts and principles discussed here have not been extensively applied to nuclear size relationships of specific organs during the metamorphic stages of amphibians. Investigators have concentrated mainly on embryos before metamorphosis and on adult tissues. This paper is concerned with these concepts and principles by way of the study of the mesonephroi of *Rana sylvatica* during metamorphosis by the use of the classical methodology of caryometry.

Materials and Methods

Two clutches of *Rana sylvatica* eggs were collected in the field and were allowed to develop in the laboratory throughout an eighty-day period. The metamorphic stages selected for study at arbitrary time intervals were identified by means of the criteria established by Taylor and Kollros (1946) for *R. pipiens*. Some of the tadpoles were carried through metamorphosis to young adulthood. Table I gives both stage and age for all animals used in this investigation. Immediately after the staging of the tadpole the body cavity was opened and the animal was immersed in Zenker-formol for fixation. After fixation the mesonephroi were removed, embedded in paraffin and sectioned either transversely or longitudinally at 6 micra. All sections were stained by the Feulgen technique after Stowell (1945).

Only the center sections of a kidney from each of the 63 animals were examined. From these sections an average of 470 camera lucida outline drawings of nuclei from the convoluted tubules were made at a magnification of 1350 × diameters. The selection of nuclei to be drawn was random. However, it was necessary to set up certain criteria as a basis for selection. The first criterion was established in the following manner. In order not to cause marked distortion in the data the outline drawing of a nucleus was made at the focus showing the greatest nuclear diameter. If the nucleus is sectioned it is impossible to determine whether or not the greatest diameter lies in the section of tissue studied or in the next succeeding section. Therefore, nuclei lying in the two cut surfaces had to be eliminated on this basis. If it was possible to focus on the tissue at a level above and at a level below the nucleus selected, then it was certain that the entire nucleus was in the section. In establishing the second criterion, the long and short diameters were determined in mm. for each drawing. From these data the nuclear volumes were calculated according to the formula for the volume of an ellipsoid. It is a fact that this volume is not often used in similar studies of biologic material because of the difficulty of determining a diameter in the third dimension. This is practically impossible in sectioned material. However, in the kidneys of *R. sylvatica* used in this study the nuclei appear more or less spherical both in cross-section and in longitudinal section. On the basis of observation, therefore, it was concluded that most of the nuclei were not markedly flattened. Any nucleus that was definitely elliptical from surface view was eliminated. Also, any nucleus that appeared obviously thin on focusing through it was also eliminated. This device is, of course, arbitrary and subjective and does not remove the difficulty of the third diameter.

TABLE I

The table shows the modal volumes in mm.³ for the peak classes, and the ratios × x/766 mm.³ where x is any one of the modal volumes and where 766 mm.³ is the lowest volume in the table

Stage	Class I nuclei	Intermediate class	Class II nuclei	Age in days
3	949(1.2)	1288(1.7)		18
4		1310(1.7)	1762.5(2.3)	24
		1290(1.7)		
5		1069(1.4)		22
		1226.5(1.6)		
		1075(1.4)		
6	766(1.0)	1105(1.4)	1378(1.8)	32
7		1225(1.6)	1364.5(1.8)	39
		1114(1.4)		
8		1231(1.6)	1400(1.8)	32
			1363(1.8)	
9		1066(1.5)	1369(1.8)	39
			1358.5(1.8)	
10		1270(1.7)	1500(1.9)	37
		1229(1.6)		
11–12		1130(1.5)	1511.5(2.0)	36
		1028.75(1.3)		
13–15	952(1.2)	1143(1.5)		36
		1061(1.4)		
16–17		1248(1.6)	1513(2.0)	39
		1138(1.5)		
18		1152(1.5)		43
		1294(1.7)		
		1109.5(1.4)		
19			1375(1.8)	45
			1369(1.8)	
			1355(1.8)	
20		1246(1.6)	1376.5(1.8)	47
			1355.5(1.8)	
21		1114(1.4)	1443(1.8)	47
			1381.25(1.8)	
22			1512(2.0)	50
			1926(2.5)	
			1811.5(2.3)	
23		1054(1.4)		57
		1334(1.7)		
		1300(1.7)		
24		1320(1.7)	1384(1.8)	57
		1160(1.5)		
*25	775.5(1.0)	1087(1.4)	1519(2.0)	80
	846(1.1)	1097.5(1.4)	1522(2.0)	
	958(1.2)		1417.75(1.8)	
			1349(1.8)	
			1433.5(1.9)	

* Young adults.

In addition, there are difficulties other than those due to diametric differences. For instance, there are a number of sources of error, not the least of which are those inherent in the investigator. The eye in seeing and the hand in drawing are not always coordinated to the same degree. There is the problem of focusing on

the maximum nuclear surface, the possibility of inaccuracy in measurement, the effect of the procedures on the nuclei themselves, and according to Merriam and Ris (1954) the probable sources of error in the camera lucida projection method. Further, there is the fact that the error due to method has been raised to a higher power in the formula for determining volumes. However, a search for absolute values and statistical certainty is not the purpose of this paper. Only a relative

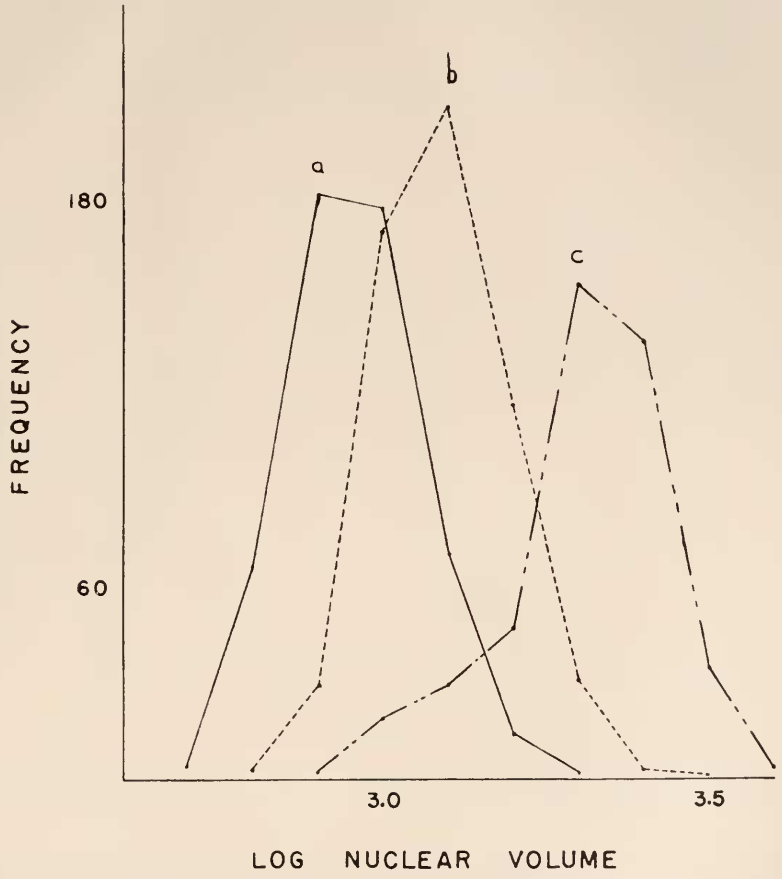

FIGURE 1. Histograms of three samples, one from each of three stages: a) stages 6, b) stage 18, c) stage 22.

value or an approximate value is sought. No attempt has been made here at statistical analysis of the data.

After determination of the nuclear volumes, these values were grouped into frequency classes and frequency curves were drawn for each one of the 63 kidneys. The modal volume for the peak class was determined for each case and they are recorded in Table I. This table shows that the lowest modal volume (766 mm.^{3}) occurs in one of the individuals at stage 6. Using this volume for comparative

purposes a ratio can be obtained for each modal volume listed. The ratios form a progression, 1 : 1.5 : 2, with nearly all intermediates between these values.

In the histograms the frequency of nuclear volumes is plotted against the class interval. Also, one third of the data were plotted by two other methods. In the one case, the long diameters of the nuclei were substituted for nuclear volumes; in the other, the logs of the nuclear volumes were plotted against frequency. The long diameters or the surface areas are frequently used as a substitute for volume

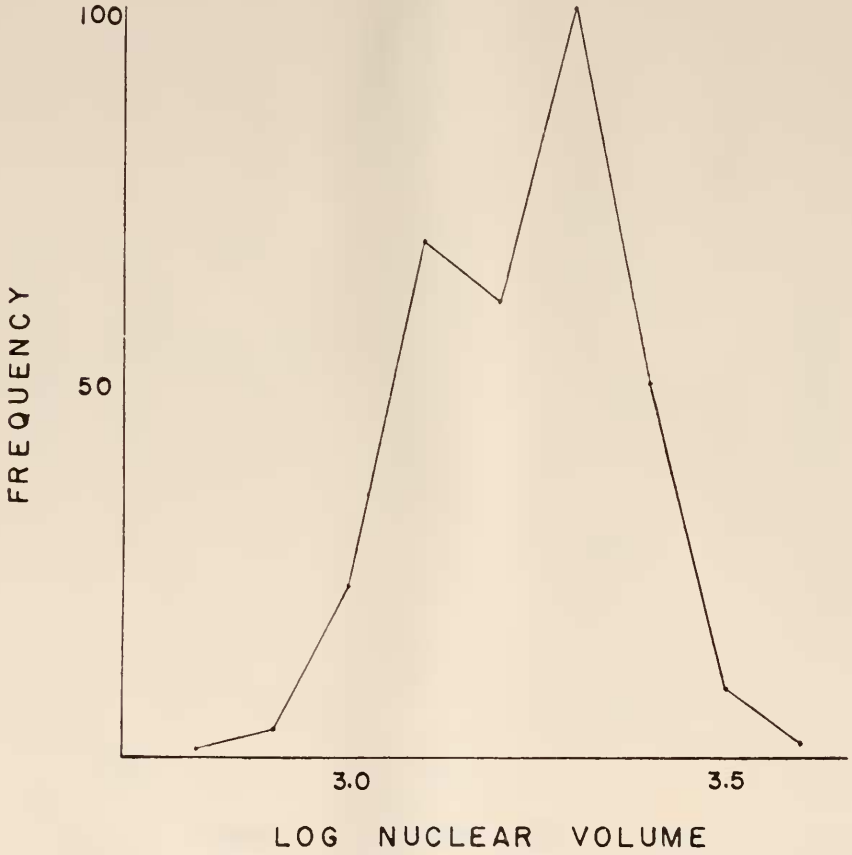

FIGURE 2. Histogram of one of the individuals at stage 24.

when the nuclei are ellipsoidal. Both values have the advantage over volume calculations since it is not necessary to multiply the error by raising the diameters to a higher power. In this study one third of the samples were selected and frequency curves were drawn using long diameters rather than volumes. In all cases the curves correspond to those obtained in histograms in which numbers were plotted against volumes. It appears, therefore, that for the data presented here there was no great advantage of one method over the other, at least in terms of the revelation of three classes of nuclei. If, however, a curve of greater symmetry is

desired then the use of logarithms of nuclear volumes has a distinct advantage over both volume and the long diameter. The logs of nuclear volumes give a more symmetrical curve than the nuclear volume itself (Figs. 1 and 2). Bucher (1954) claimed that the logarithmic system of classification is better adapted to a mathematical analysis of the curve and that it is the only valid system from a biological and statistical viewpoint. On the other hand, it has been stated (Bonner and Eden, 1956) that a mathematical analysis of the curve is not especially helpful for comparative purposes except in those restricted cases where there is additional information about cell or nuclear growth.

Finally, the geometric mean of the nuclear volumes was calculated for each kidney for purposes of plotting the logarithmic growth curve (Fig. 3). The geo-

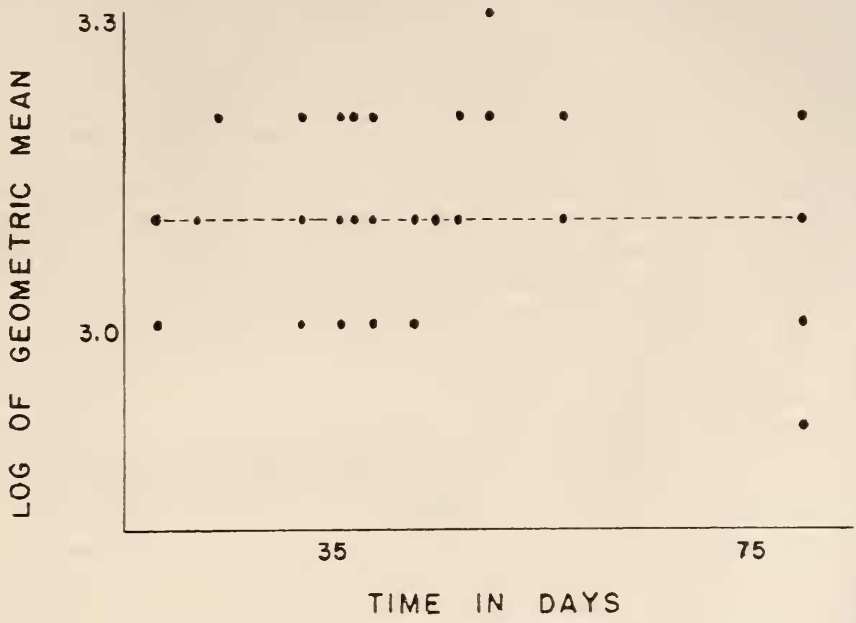

FIGURE 3. Logarithmic growth curve. Each point plotted represents from one to four individuals. Thus, at 80 days the 10 young adults are represented in the four points.

metric mean is subject to all those errors inherent in the method and is, like the nuclear volume, an approximation.

OBSERVATIONS

The frequency distribution curves of all but one of the kidneys studied show a single peak and are therefore unimodal. There is one bimodal curve (Fig. 2) in which the nuclear volumes give two peak classes. In all cases, when the modal volumes (Table I) calculated for these peaks are arranged in a series they form a progression in the ratio 1 : 1.5 : 2. Therefore, the histograms reveal two major classes and one intermediate class (Fig. 1).

In Class I the nuclei are believed to have the diploid value characteristic of post-

mitotic nuclei. Following division there is, possibly, a period of interphasic growth of the chromosomes during which the nuclear volumes increase to the size of the intermediate class. This may be followed by a second period of interphasic growth during which the nuclear volume becomes twice that of the diploid nucleus. Nuclei of Class II may, therefore, be tetraploid and ready for division. All intermediate values shown in Table I are interpreted hypothetically as intermediate classes which would be expected to appear during the two interphasic growth periods. The three peak classes are shown in Figure 1 where three of the 63 histograms were selected for illustrative purposes. It is to be noted that in both Figures 1 and 2 the log nuclear volume has been substituted for nuclear volume.

Examination of Table I, with the assumptions noted above in mind, will reveal that modal volumes of Class I are rare, and that the intermediate class occurs most frequently. It is perhaps significant that whereas 30% of the young adults have modal volumes of Class I, only about 5% of the tadpoles do. The number of kidneys in the tetraploid class (Class II) are most numerous through stages 7–10, 19–22 and in the young adults. In stage 7 through 10 and in the young adults 50% of the kidneys sampled give peak classes in the tetraploid range, while in stages 19–22 approximately 83% of the samples are in this range. It appears, therefore, that the greatest periods of mitotic activity occur within the stages indicated.

The logarithmic growth curve (Fig. 3) also serves to point out some of these relationships. The growth curve is a straight line drawn through points which represent the intermediate class described above. The scatter above and below the line is due to the occurrence of samples which give peak classes in the Class I and in the Class II range. Obviously, however, there are no marked increases or decreases in growth that can be correlated with age. All nuclear classes may be found in the kidneys of tadpoles which are of the same age, just as all nuclear classes may be found in the kidneys of tadpoles in the same stage (Table I). Nevertheless, a rough correlation can be made for stages 19–22. The animals in these stages are 45–50 days old. At 45–50 days the scatter is all above the line of growth indicating, perhaps, increased mitotic activity at this time.

A single bimodal curve was revealed in one of the individuals at stage 24 (Fig. 2). The two peaks correspond to the intermediate class and to Class II nuclei. Other instances of bimodality were suggested in the histograms. But in all cases except the one they were not revealed by either of the other graphing techniques, and are considered insignificant. With the one exception all histograms regardless of the technique were unimodal. However, when nuclear volumes are used in the histograms the curves show a marked asymmetry and are skewed to the right. This marked asymmetry disappears when the logs of nuclear volumes are used in place of the nuclear volume itself.

Discussion

The commonly accepted explanation for the appearance of the different nuclear classes in the frequency distribution curve is that for some nuclei growth in size is arrested at one or another of the growth stages, resulting in an accumulation of nuclei in the stage at which growth ceased. The tissue, as a result, carries accumulations of nuclei of differing sizes which are responsible for the peaks in the curve. Nuclei with volumes in the Class II range and greater, represent tetraploid

nuclei and the higher degrees of polyploidy, while those with volumes Class I–Class II represent interphasic sizes between successive mitoses. Hertwig (1939) in measuring nuclei from the early cleavage stages of mouse ova demonstrated that at each mitosis the nuclear volume was halved and he postulated that these volume changes could be correlated with similar changes in the genome. Alfert (1950), who studied cleavage stages in the mouse by photometric analysis of DNA content, reported doubling of DNA amounts in the interphase preceding mitosis. The significance of this observation derives from the fact that there is considerable evidence, obtained principally by the use of quantitative techniques, which suggests that DNA is either identical with the genic material or is at least closely associated with it. The same quantitative distributional patterns hypothesized by cytogeneticists for genic material can be directly applied to distributional patterns hypothesized for DNA. If this generalization is applied here, then a relationship must exist between nuclear volume and DNA content. Swift (1950) and Truong and Dornfeld (1955), using the photometric method for DNA determination in combination with caryometry on different animal tissues, showed that there is a definite relationship between DNA amount and nuclear size. Both volumes and DNA amounts fall into the ratio 1 : 2 : 4. In stable adult tissues (Swift, 1950, 1953; Pollister, Swift and Alfert, 1951) the DNA classes are clearly demarcated with little or no overlapping, while in actively dividing tissues there may be considerable overlapping because of the appearance of intermediate classes. The intermediate classes are due to gradual DNA synthesis during interphase. When the DNA reaches tetraploid level the nucleus enters prophase. After quantitative division of DNA at anaphase the diploid (Class I) amount of DNA is restored in the telophase nuclei. The cycle of DNA reduplication then repeats itself. Similarly, when labile tissues are studied by means of nuclear volume determinations, intermediate classes are revealed (Schreiber and Angeletti, 1940; Schreiber, 1949). In these cases the volume changes are interpreted as resulting from reduplicating phenomena in the genome.

In the kidneys of *R. sylvatica* used in this study there were no DNA determinations. Interpreting the results, however, on the basis of the above discussion it is possible to conclude that the data reveal the typical nuclear classes of a mitotic cycle, and a whole series of intermediate classes between Class I and Class II nuclei. The histograms (Figs. 1 and 2) show considerable overlapping. The overlapping is so marked that when the individuals are grouped and a combined histogram of all 63 kidneys is drawn, only one nuclear class is revealed. The modal value of this class falls approximately half way between Class I and Class II. It is generally believed that the increase in nuclear volume is rhythmic and that periods of rapid growth alternate with periods of relative inactivity. It is not possible from the results of this investigation to determine conclusively whether rhythmic growth as opposed to continuous growth is present here or not. For one thing, the degree of overlapping precludes any affirmative assumption concerning the presence of rhythmic growth. Not only are there no sharp demarcations between peaks suggesting discontinuity but there are also few positive correlations between the peak class and the stage and/or age of the animal. Secondly, the mere fact of the existence of a series of nuclear classes in a tissue does not imply that the details of individual nuclear growth are known, for normal frequency distributions may be

obtained not only from actively dividing tissues but from stable tissues as well (Bonner and Eden, 1956). However, the low incidence of Class I nuclei in the mesonephros of *R. sylvatica* may be due to a period of very rapid growth immediately following mitosis, so that Class I nuclei occur only transitionally. There is the possibility that the large percentage of nuclei in the intermediate class accumulate in this class due to a decrease in nuclear activity. Nuclei from this reservoir may be released gradually into the second growth period, at the end of which there is an accumulation of Class II nuclei. Schreiber and Angeletti (1940) found that in the mitotic cycle of hepatic cells of the carp there is a rhythmic increase and decrease in nuclear volume which can be correlated with the stage of development. In the kidneys of *Rana sylvatica* there is no such clearcut correlation. All nuclear classes may be present irrespective of the stage of development or of the age of the animal (Fig. 3). The developmental pattern in the kidney does not, therefore, reflect the details of nuclear growth. However, the percentage of Class II nuclei is greatest at stages 7–10, 19–22 and in the young adults. This suggests not only the possibility of three waves of mitotic activity but also a rhythmical growth pattern.

The nuclear size increase recorded here may not be associated with the duplicating process of the genome or with an increase in DNA. It has been discovered that in a given tissue, while DNA remains constant per nucleus, the protein content and the nuclear size show corresponding increases (Alfert, 1950; Biesele, 1944; Schrader and Leuchtenberger, 1950; Leuchtenberger and Schrader, 1951). Further, the chromosomal volume may increase by protein synthesis without an accompanying morphological change in nuclear size (Biesele, 1944). Also the nuclear volume may even be reduced as a result of water loss rather than by a change in the genome (Krantz, 1947). Furthermore, differences in nuclear size may have to do with nutritional differences that do not affect chromosomal size (Montgomery, 1910). Even in a normally dividing tissue there may be a size difference due to some factor other than that which stimulates cell activity. For instance, the age of the animal may affect cell or nuclear size quite independently of a tendency toward compensatory hypertrophy (Buchner and Glinos, 1950).

In view of these considerations a number of possibilities come to mind in regard to the increase in nuclear size observed here. This increase has been attributed to mitotic activity, or to an increase in chromosomal content. It is conceivable that some of this increase is due to imbibition or to an increase in the osmotic concentration consequent upon the synthetic activity of the chromosomes. Or synthetic activity in the interphase nucleus may alter the nuclear membrane, causing osmotic changes. This may effect an increase in nuclear volume which is not exactly paralleled by an increase in the volume of the chromosome. As a result nuclear volumes may vary to such an extent that there is considerable overlapping of volume classes in frequency curves. The increase in size of a nucleus may be partially due to mechanical pressure (Teir, 1949). In addition to the factors postulated above it is known that a morphological increase in size may be due to compensatory hypertrophy following such operations as unilateral nephrectomy (Sulkin, 1949) or partial hepatectomy (Sulkin, 1943), to hormonal agents like oestrone (Salvatore, 1950; Alfert and Bern, 1951; Schreiber, 1954), or to agents which stimulate chromosomal activity such as thiouracil (Roels, 1954), alloxan (Diermeier *et al.*, 1951) and colchicine (Bucher, 1951; Fankhauser, 1952). The administration of

any one of these agents may be followed by morphological changes in the nucleus, but need not necessarily be the direct cause of that change. Undoubtedly the size increase involves and is indicative of phenomena which are very complex due to the complexity of factors forming the cytoplasmic and nuclear ecology.

Some of the agents mentioned may not only be causative factors in increasing nuclear size but they may also induce polyploidy. Fankhauser (1952) by the use of colchicine induced endopolyploidy in embryos of the axolotl. Some of these embryos were originally diploid. It is generally known that polyploidy does occur in normal diploid animals. This condition, known as polysomaty or endopolyploidy, has been reported in the renal tubules of *Leptodactylus,* a South American frog, by Schreiber and Melucci (1949) and in the renal tubules of *Cyclorana,* the Australian desert frog by Dawson (1948). Polysomaty does not occur here in the kidneys of *R. sylvatica.* Twenty-six of the kidneys give modal volumes belonging to Class II. Conceivably, some of the nuclei in this peak class might be true polyploids with twice the diploid number of chromosomes rather than interphasic cells approaching a proliferative stage. There is no way of determining this, however, by the method used here. The histograms gave no peak classes at higher than the hypothetical tetraploid level. Possibly, some of the very large nuclei are octoploids. If so they are so rare that there is no great accumulation at this level and therefore the higher peaks are not obtained. In tissues showing a relatively high incidence of polyploid cells the frequency increases with age (Swartz, 1956). Polyploid cells may be absent then in the tadpole kidneys and in young adults and may appear only in older animals. However, it is now a well known fact (*cf.* Fankhauser, 1945) that polyploidy does occur in tadpole tissues. It can only be concluded, therefore, that there are few if any polyploid cells in the mesonephroi of these *R. sylvatica.* This is consistent with the general opinion that the incidence of polyploidy in kidney tissues is relatively rare, even though it is known to occur in those instances cited above.

The concept of endopolyploidy, or any concept of variation in chromosome number, renders untenable the hypothesis that for a given species the chromosome number is the same for all of the somatic cells. The "constancy hypothesis" has, therefore, been revised on the assumption that DNA is somehow related to the genic material. The constancy of DNA per chromosome set, first proposed by Boivin, Vendrely, and Vendrely (1948), and by Ris and Mirsky (1949), is supported by the work of Pollister and his school (*cf.* Pollister, Swift and Alfert, 1951). As pointed out above, increased metabolic activity in the chromosomes as shown by DNA synthesis is accompanied by an increase in nuclear size. If this increase is always proportionate to DNA increase then a constant relationship should exist between DNA content per chromosome set and nuclear size. Obviously, such a constant relationship does not exist. The phenomenon of variation in nuclear size may very well be a secondary event which may or may not be associated with DNA content. In terms of "constancy," assumptions concerning chromosome number and probable DNA content cannot always be made from nuclear volume data. There is also a certain amount of evidence which calls into question the constancy of DNA per chromosome set. Roels (1954) working with rat thyroid and Diermeier *et al.* (1951) with the liver of alloxan diabetic rats have presented evidence to indicate that the DNA content of a cell may vary with its degree of functional activity. Pasteels and Lison (1950) concluded that the DNA content of certain rat tissues is lower than the diploid value because of chromatin diminution. Though this particular evi-

dence has been questioned (Alfert and Swift, 1953), chromatin diminution is known to occur in *Ascaris* (Boveri, 1904) and in some other forms (see the brief review of Tyler, 1955.) Also, Marshak and Marshak (1955) discuss negative DNA reactions in the *Arbacia* egg. Their evidence, however, has also been questioned (*cf.* Burgos, 1955; Marshak and Marshak, 1956). The exceptions to DNA constancy still raise a question in regard to the chemical nature of the gene, and they must be kept in mind when interpreting data having to do with nuclear volumes.

The occurrence of mitotic activity in the mesonephroi herein investigated indicates that during metamorphosis the kidney has some cells that are undifferentiated. At least physiologic inactivity in terms of renal function can be postulated for the proliferating cells. Some of the non-dividing cells, however, must be already manifesting renal activity. If so then the nuclear sizes may be indicative of a differentiating process. The fact that nuclear size and characteristic cell structure are intimately related is most dramatically demonstrated in insects. For example, in certain honeybee tissues (Merriam and Ris, 1954) the nuclear volume not only increases with age but there is a direct correlation between the nuclear size and secretory activity. Of equal importance is the fact that in certain mammalian tissues such as rat liver the increase in numbers of large nuclei closely parallels the histological and functional development of the organ (Sulkin, 1943; McKellar, 1949). A somewhat similar relationship has been demonstrated for a species of the slime mold, *Dictyostelium* (Bonner, 1957). In this mold, the stage of development and the early stages of differentiation are reflected in both the cell size and in the nuclear size which is characteristic of the particular stage. Further, the most active cells of this slime mold are located at the anterior end of a migrating sausage-shaped mass. The active cells are also the larger and are, according to Bonner, responsible for important morphogenetic effects which end in the formation of the fruiting body. Another parallel between cell size and differentiating activity may be seen in the developing sea urchin egg. It is well known that the micromeres formed during the cleavage stages of the sea urchin egg play an important role in morphogenesis in that they induce vegetal differentiation. McMaster (1955), working with *Lytechinus,* has shown by photometric determinations of DNA amounts in the cleavage cells that the lowest DNA amounts are in the micromeres. It is possible that the differentiating effects of these cells may be due to the lower DNA values. Once again, therefore, the point to be made here is that cell size is associated with a differentiating process. Progressive differentiation may also be associated with quantitative differences in DNA amounts in amphibians. Moore (1952), in working with haploid and diploid tissues of *Rana pipiens* embryos, found that the range of DNA values in the forebrain was greater in the 7-day embryos than in the 11-day embryos. She found no correlation between mitotic activity and the amount of DNA. In explaining this she discusses a possible correspondence between DNA amount and differentiation. DNA may decrease with age and with the maturation of the tissue until it reaches some more or less constant value. If this is so then nuclear volumes may also be smaller in adults than in embryos. Perhaps increased differential activity accounts for the larger percentage of Class I nuclei recorded (Table I) here for the young adults of *Rana sylvatica.*

At histological maturation differentiation may be influenced by polysomaty. Polyploid cells at the time of differentiation may return to a lower value by some process such as reduction mitosis (Huskins, 1948). The genic segregation which

accompanies the reductional division may produce cells whose differentiating potential is quite different. This interpretation puts stress on ploidy as a causative factor in differentiation. In considering polyploid organisms it is known that generally they differentiate normally and effects of ploidy, such as larger nuclear or cell size, are considered secondary. Mather (1948) has insisted that duplication of the chromosome number is not the cause of differentiation. The fate of the cell, he concluded, is dependent not so much on the nucleus which in most cases is diploid in sexually reproducing animals or plants, but on a cytoplasm which is inherited from the past. The action of the nucleus is effective only because the cytoplasm has changed at each step along the way. At each division the nuclei are quantitatively and qualitatively alike but each one inherits a portion of cytoplasm, one portion of which cannot be equated with the other. If polyploidy occurs it is probably in response to the cytoplasm.

In conclusion, the difficulties of interpreting nuclear size relationships are many and varied. Not only are the techniques used for such studies possessed of their own difficulties but nuclear size itself may be altered by a number of conditions. Size changes may be physiological, mitotic, or due to different degrees of heteroploidy. If genic changes are involved then these changes may be correlated with alterations in DNA amounts. The results strongly suggest that DNA is the genic material. But the fact of the matter remains that the gene is a biological concept, an abstraction, known only in its effects. The exact relationship between the gene and DNA is still unknown. A nuclear size change, itself, may be a phenomenon occurring coincidentally with differentiation, as if there were two progressions, one of which functions as the cause or the effect of the other. Or instead, all the events involved may converge and commingle as an expression of a single phenomenon reaching peak expression in the differentiated living cell. Increases or decreases in nuclear size are morphological and physiological events which take part in this convergence.

Summary

1. The mesonephroi from 63 *Rana sylvatica* tadpoles and young adults were studied in sectioned material by means of a caryometric method for determination of nuclear volumes. Frequency histograms drawn from the data reveal three peak classes which, when arranged in series, give the progression, 1 : 1.5 : 2. These results are interpreted as being due to an interphasic growth preceding mitosis.

2. The results are discussed in terms of morphological increase and decrease in nuclear size, increase and decrease of DNA values, polyploidy, the "constancy" hypothesis, physiological activity and histological differentiation.

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